面に表示してもよい）。また、ＭＲＩ装置の実物にトラックボールなどの入力装置が付属している場合には、それも画面内に操作可能に表示することが好ましい。さらに、テーブルの天板位置を表すＬＥＤ（Light-Emitting Diode）ディスプレイ等を模した画像を更に表示してもよい。

【００４３】

ステップａ３２では、操作者は、撮影シミュレーション用ウェブページ画面Ｇ３０の操作卓表示領域Ｒ３１上でのボタン操作をマウス操作等により行い、撮影に必要なスキャンパラメータを設定する。このスキャンパラメータは、前記スキャンパラメータ等表示領域Ｒ３２に反映される。

ステップａ３３では、顧客側端末は、ネットワーク１を介して、ホストサーバ装置２００に対し、スキャンパラメータを送信する。

【００４４】

ステップｓ３１では、ホストサーバ装置２００は、通信回線１０２を介して、前記スキャンパラメータを、演算装置２０１に転送する。

ステップｐ３１では、演算装置２０１は、前記スキャンパラメータを受信する。

【００４５】

ステップａ３４では、操作者により操作シミュレーション用ウェブページ画面Ｇ３０の操作卓表示領域Ｒ３１上の「Scan Start」ボタンがクリックされたら、ネットワーク１を介して、ホストサーバ装置２００に対し、撮影開始要求を送信する。

【００４６】

ステップｓ３２では、ホストサーバ装置２００は、通信回線１０２を介して、前記撮影開始要求を、演算装置２０１に転送する。

ステップｐ３２では、演算装置２０１は、前記撮影開始要求を受信する。

【００４７】

ステップｐ３３では、演算装置２０１のダミーイメージ選択部２１Ｄは、前記スキャンパラメータを解析し、撮影を仮想的に行った場合に生成されると予測されるイメージに最も近いダミーイメージを選択する。

ステップｐ３４では、演算装置２０１は、通信回線１０２を介して、ホストサーバ装置２００に対して、前記選択したダミーイメージを送信する。

【００４８】

ステップｓ３３では、ホストサーバ装置２００は、ネットワーク１を介して、前記ダミーイメージを、顧客側端末に転送する。

【００４９】

ステップａ３５では、顧客側端末は、前記ダミーイメージを受信する。

ステップａ３６では、撮影シミュレーション用ウェブページ画面の動作再現画像表示領域に、前記動作再現画像を表示する。例えば、図１６の撮影シミュレーション用ウェブページ画面Ｇ３０’に示すように、ダミーイメージ表示領域Ｒ３３にダミーイメージが表示される。

ステップａ３７では、シミュレーションを終了するならば操作／撮影シミュレーション処理を終了し、シミュレーションを続ける（操作シミュレーションと撮影シミュレーションのどちらかを続けて行う）ならば上記ステップａ２１に戻る。

【００５０】

なお、操作／撮影シミュレーションを行い得る医用画像診断装置の機種が複数ある場合には、例えばプルダウンメニュー形式で表示された候補のうちから操作者が選択した機種名を、前記操作シミュレーション用ウェブページ画面Ｇ２０，Ｇ２０’（図１２，図１３参照）および前記撮影シミュレーション用ウェブページ画面Ｇ３０，Ｇ３０’（図１５，図１６参照）内に表示することが好ましい。

【００５１】

以上の第２の実施形態にかかる医用画像診断装置の操作／撮影シミュレーションシステム２０００によれば、顧客が自己の端末（図１の１１～１４）に表示された操作シミュレーション用ウェブページ画面Ｇ２０や，操作シミュレーション用ウェブページ画面Ｇ３０上で医用画像診断装置の操作や撮影を疑似体験して操作性の良否などを直感的に評価できる。

【００５２】

－第３の実施形態－

図１７は、本発明の第３の実施形態にかかるノートパソコン３００およびＣＤ－ＲＯＭ（Compact Disk - Read Only Memory）３０１を示す構成図である。

このＣＤ－ＲＯＭ３０１には、設置シミュレーションプログラム３１Ａと，操作シミュレーションプログラム３１Ｂと，撮影シミュレーションプログラム３１Ｃとが記録されている。

【００５３】

前記設置シミュレーションプログラム３１Ａは、ＭＲＩ装置などの医用画像診断装置の設置空間の大きさを入力させるディメンション入力ステップと，前記医用画像診断装置を前記設置空間内に仮想的に設置したときの設置イメージを生成する設置イメージ生成ステップと，前記設置イメージを表示する設置イメージ表示ステップとを、ノートパソコン３００の実行コード形式で記述したものである。すなわち、ノートパソコン３００で設置シミュレーションプログラム３１Ａを実行することで、スタンドアロン環境で、上記第１の実施形態にかかる設置シミュレーションを行うことが可能となる。

【００５４】

前記操作シミュレーションプログラム３１Ｂは、医用画像診断装置の操作部を模した画像上で与えられた操作要求または前記操作部と同等の機能を有する入力装置を介して与えられた操作要求を受け付ける操作要求受付ステップと，前記操作要求に応じた医用画像診断装置の動作を擬似的に再現した動作再現画像を生成する動作再現画像生成ステップと，前記動作再現画像を表示する動作再現画像表示ステップとを、ノートパソコン３００の実行コード形式で記述したものである。すなわち、ノートパソコン３００で操作シミュレーションプログラム３１Ｂを実行することで、スタンドアロン環境で、上記第２の実施形態にかかる操作シミュレーションを行うことが可能となる。

【００５５】

前記撮影シミュレーションプログラム３１Ｃは、医用画像診断装置の操作部を模した画像上で与えられた撮影要求または前記操作部と同等の機能を有する入力装置を介して与えられた撮影要求を受け付ける撮影要求受付ステップと，前記撮影要求に応じた撮影を前記医用画像診断装置が仮想的に行った場合に得られる医

用イメージを表示する医用イメージ表示ステップとを、ノートパソコン３００の実行コード形式で記述したものである。すなわち、ノートパソコン３００で撮影シミュレーションプログラム３１Ｃを実行することで、スタンドアロン環境で、上記第２の実施形態にかかる撮影シミュレーションを行うことが可能となる。

【００５６】

以上の第３の実施形態にかかるノートパソコン３００およびＣＤ－ＲＯＭ３０１によれば、スタンドアロン環境で上記第１，第２の実施形態にかかる設置／操作／撮影シミュレーションを行うことが可能なので、例えば営業担当者が顧客元に出向いて営業活動を行う際に、医用画像診断装置の設置性や操作性を説得力を持ってプレゼンテーションできるようになる。

【００５７】

なお、上記ＣＤ－ＲＯＭ３０１の代わりに、ＦＤ（Floppy Disk）や，ＭＯ（Magneto-Optical）ディスクや，ＤＶＤ（Digital Versatile Disk）や，ＰＤ（Phase-change Disk）などの可搬型記録媒体を使用してもよい。また、ノートパソコン３００の内蔵ハードディスクに、設置／操作／撮影シミュレーションプログラム３１Ａ，３１Ｂ，３１Ｃをインストールしてもよい（この場合には、可搬型記録媒体から各プログラムを読み出す必要がなくなる）。

【００５８】

【発明の効果】

本発明の設置シミュレーション方法、システム、プログラムおよび記録媒体によれば、指定した大きさの設置空間内に医用画像診断装置を設置した状況を表した設置イメージを自動表示できるようになる。

また、本発明の操作シミュレーション方法、システム、プログラムおよび記録媒体によれば、操作部を模した画像または入力装置を用いて、医用画像診断装置の操作を擬似体験できるようになる。

さらに、本発明の撮影シミュレーション方法、システム、プログラムおよび記録媒体によれば、操作部を模した画像または入力装置を用いて、医用画像診断装置による撮影を擬似体験できるようになる。

【図面の簡単な説明】

【図１】

第１の実施形態にかかる医用画像診断装置の設置シミュレーションシステムを示すブロック図である。

【図２】

図１の設置シミュレーションシステムにおけるホストサーバ装置および前記演算装置の構成ブロック図である。

【図３】

設置シミュレーション処理を示すフロー図である。

【図４】

図３の続きのフロー図である。

【図５】

設置シミュレーション用ウェブページ画面の例示図である。

【図６】

設置パラメータの指定方法を示す説明図である。

【図７】

エラーメッセージ画面の例示図である。

【図８】

設置シミュレーション用ウェブページ画面の別の例示図である。

【図９】

第２の実施形態にかかる操作／撮影シミュレーションシステムのホストサーバ装置および演算装置の構成ブロック図である。

【図１０】

操作／撮影シミュレーション処理を示すフロー図である。

【図１１】

図１０の続きのフロー図である。

【図１２】

操作シミュレーション用ウェブページ画面の例示図である。

【図１３】

操作シミュレーション用ウェブページ画面の別の例示図である。

【図１４】

時間短縮倍率調整画面の例示図である。

【図１５】

操作シミュレーション用ウェブページ画面の例示図である。

【図１６】

操作シミュレーション用ウェブページ画面の別の例示図である。

【図１７】

第３の実施形態にかかるノートパソコンおよびＣＤ－ＲＯＭを示す構成図である。

【図１８】

医用画像診断装置の従来の説明画面を示す説明図である。

【図１９】

図１８の説明画面のリンク先の詳細説明画面を示す説明図である。

【符号の説明】

１０００　医用画像診断装置の設置シミュレーションシステム

２０００　医用画像診断装置の操作／撮影シミュレーションシステム

１　　ネットワーク

１０Ａ　通信部

１０Ｂ　入力部

１０Ｃ　出力部

１０Ｄ　設置シミュレーション用ウェブページ格納部

１１，１２，１３，１４　顧客側端末

１１Ａ　接続部

１１Ｂ　鳥瞰図生成部

１１Ｃ　正面図生成部

１１Ｄ　平面図生成部

１１Ｅ　視点変更クリックポイント決定部

２０Ｄ　操作シミュレーション用ウェブページ格納部

２０Ｅ　撮影シミュレーション用ウェブページ格納部

２１Ｂ　動作再現画像生成部

２１Ｃ　ダミーイメージ格納部

２１Ｄ　ダミーイメージ選択部

１００，２００　ホストサーバ装置

１０１，２０１　演算装置

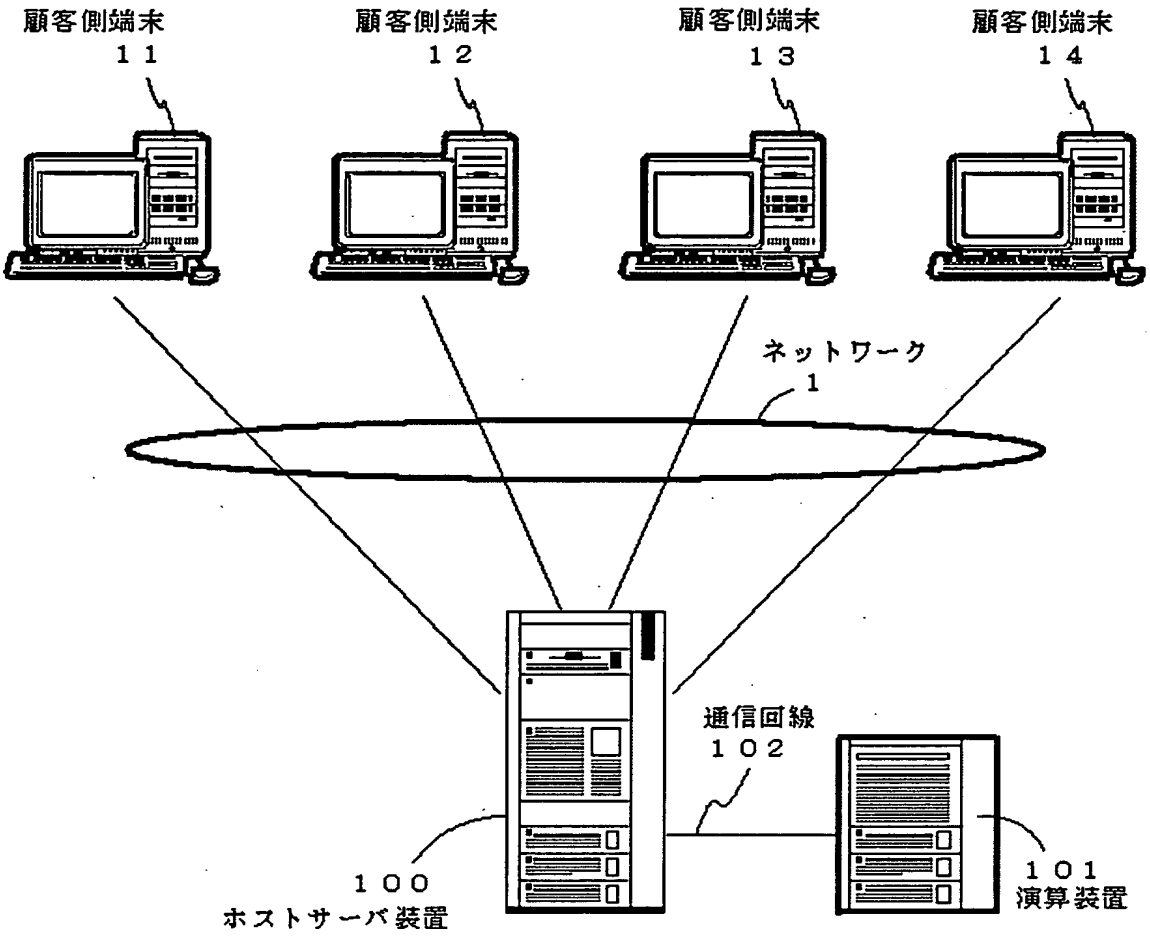
１０２　通信回線

１１０　設置イメージ生成部

【書類名】　図面

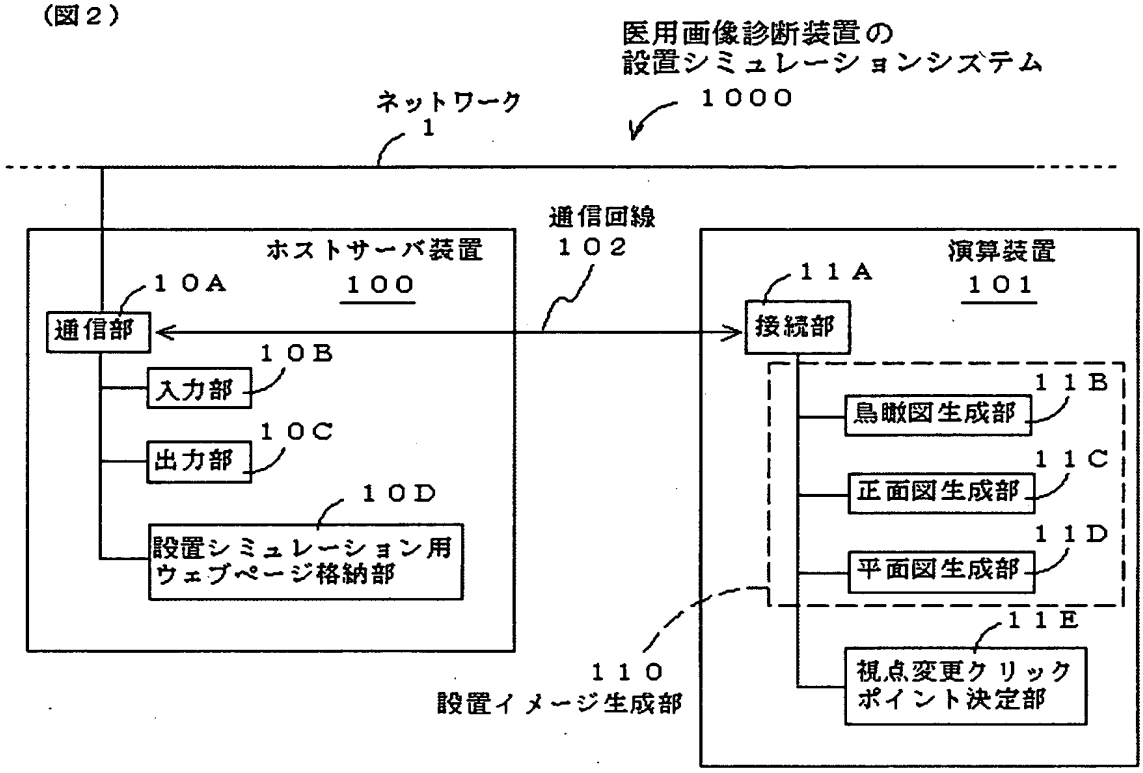
【図１】

（図１）　医用画像診断装置の
設置シミュレーションシステム
１０００

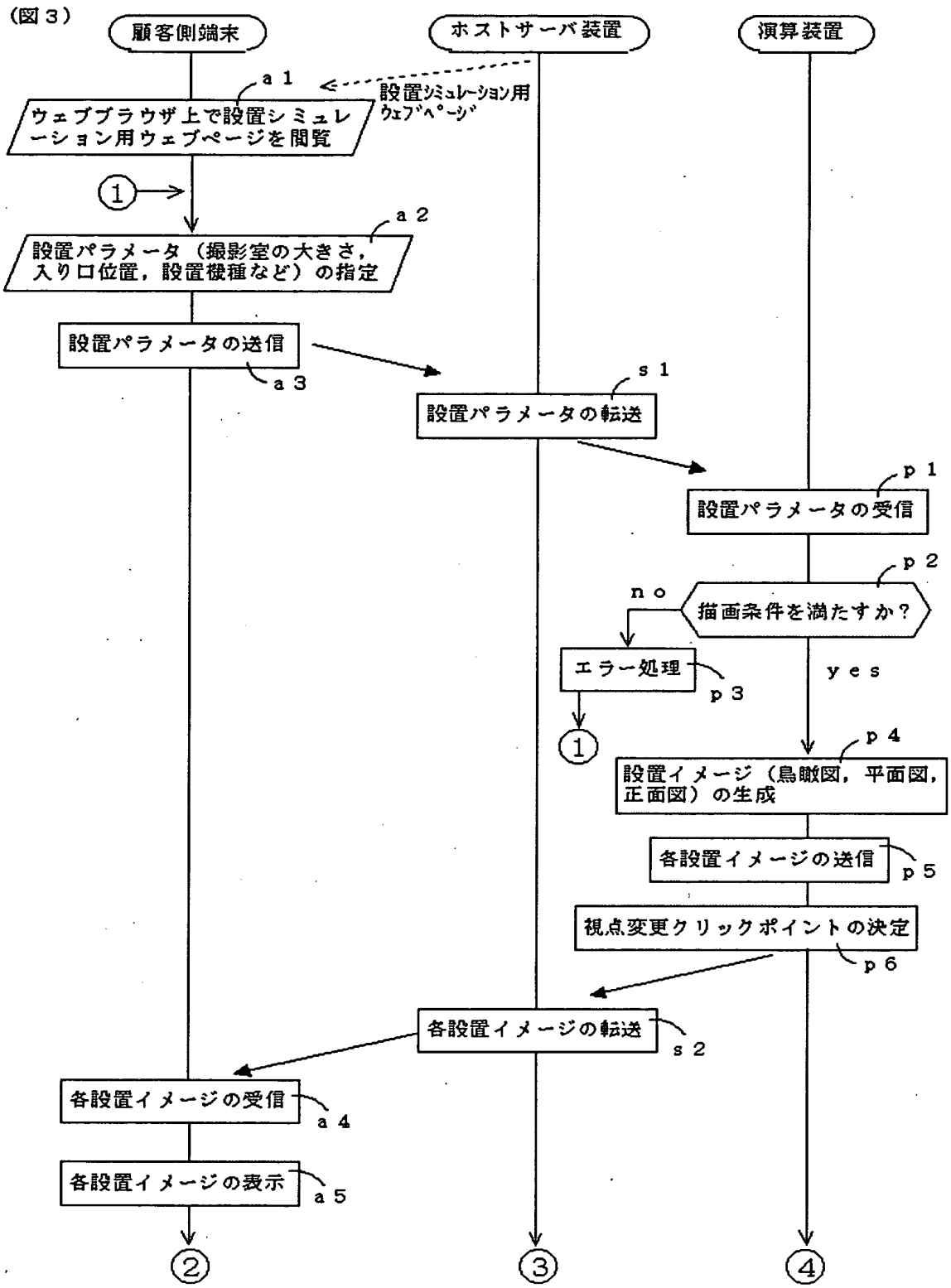


【図２】

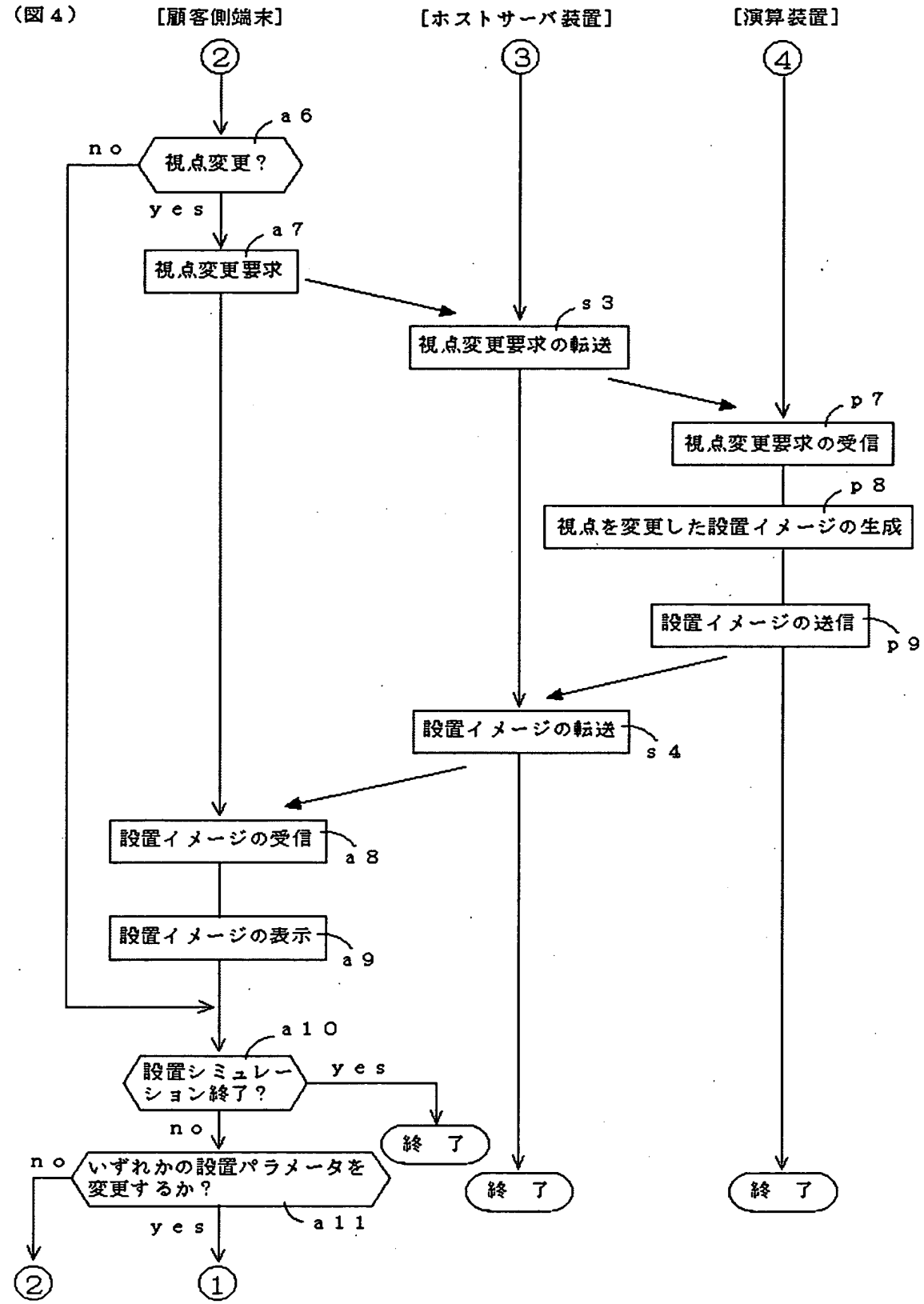
（図２）


医用画像診断装置の
設置シミュレーションシステム
1000
ネットワーク
1
通信回線
102
ホストサーバ装置
100
10A
通信部
10B
入力部
10C
出力部
10D
設置シミュレーション用
ウェブページ格納部
演算装置
101
11A
接続部
11B
鳥瞰図生成部
11C
正面図生成部
11D
平面図生成部
11E
視点変更クリック
ポイント決定部
110
設置イメージ生成部

【図３】

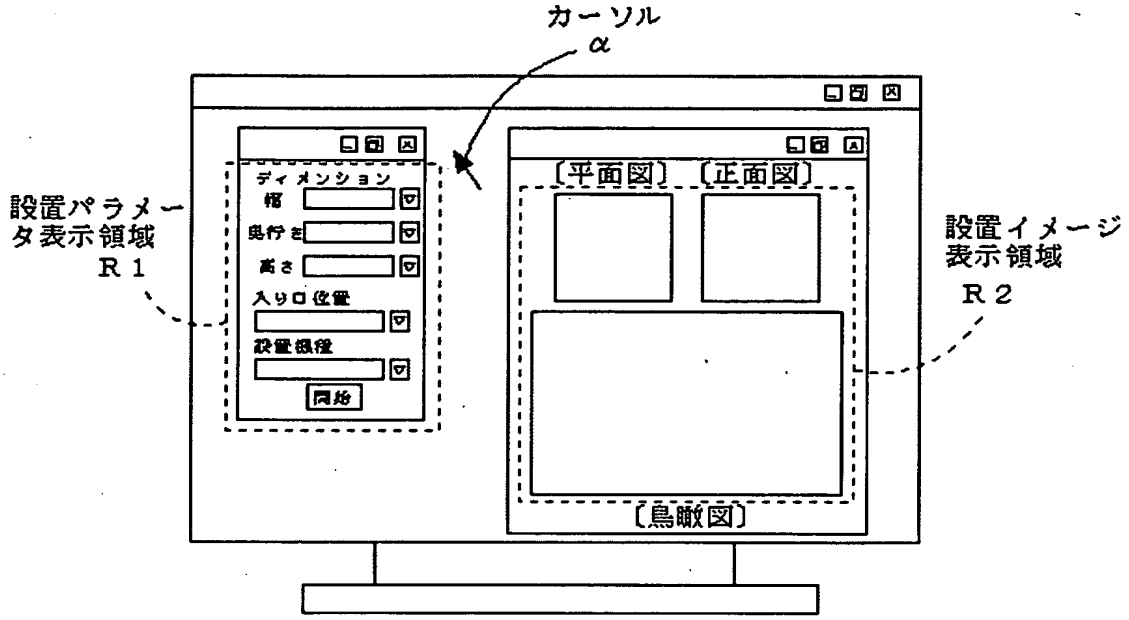

(図３)
顧客側端末
ホストサーバ装置
演算装置
a 1
ウェブブラウザ上で設置シミュレーション用ウェブページを閲覧
設置ｼﾐｭﾚｰｼｮﾝ用ｳｪﾌﾞﾍﾟｰｼﾞ
①
a 2
設置パラメータ（撮影室の大きさ，入り口位置，設置機種など）の指定
設置パラメータの送信
a 3
s 1
設置パラメータの転送
p 1
設置パラメータの受信
p 2
描画条件を満たすか？
no
yes
エラー処理
p 3
①
p 4
設置イメージ（鳥瞰図，平面図，正面図）の生成
各設置イメージの送信
p 5
視点変更クリックポイントの決定
p 6
各設置イメージの転送
s 2
各設置イメージの受信
a 4
各設置イメージの表示
a 5
②
③
④

【図４】


（図４）
［顧客側端末］
［ホストサーバ装置］
［演算装置］
②
③
④
a６
視点変更？
ｎｏ
ｙｅｓ
a７
視点変更要求
ｓ３
視点変更要求の転送
ｐ７
視点変更要求の受信
ｐ８
視点を変更した設置イメージの生成
設置イメージの送信
ｐ９
設置イメージの転送
ｓ４
設置イメージの受信
a８
設置イメージの表示
a９
a１０
設置シミュレーション終了？
ｙｅｓ
ｎｏ
終　了
終　了
終　了
ｎｏ
いずれかの設置パラメータを変更するか？
ｙｅｓ
a１１
②
①

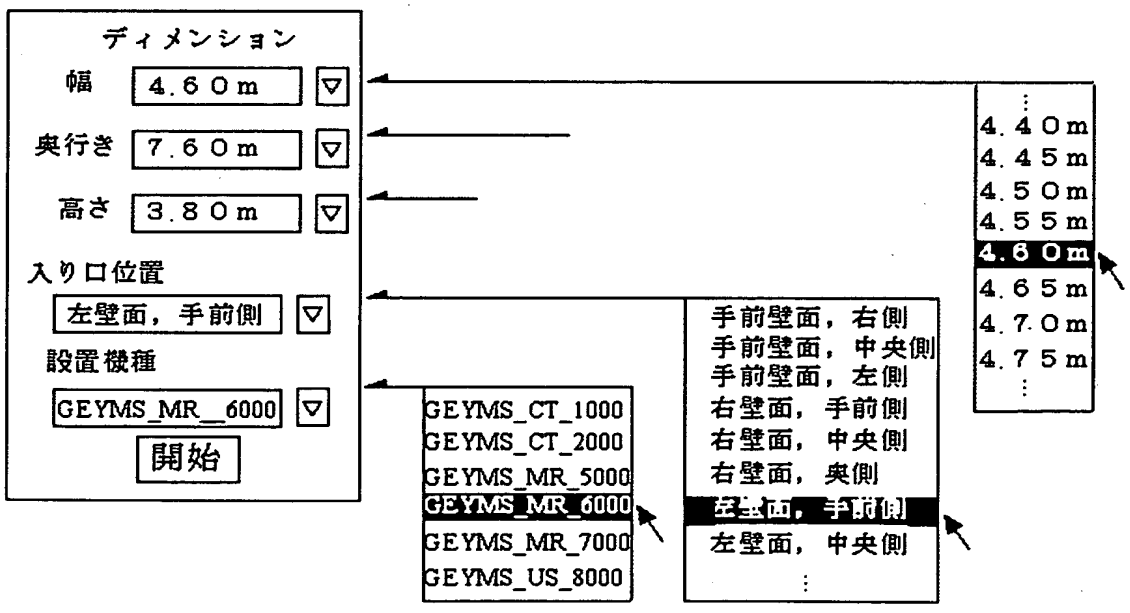
【図５】

（図５）　設置シミュレーション用ウェブページ画面
Ｇ１



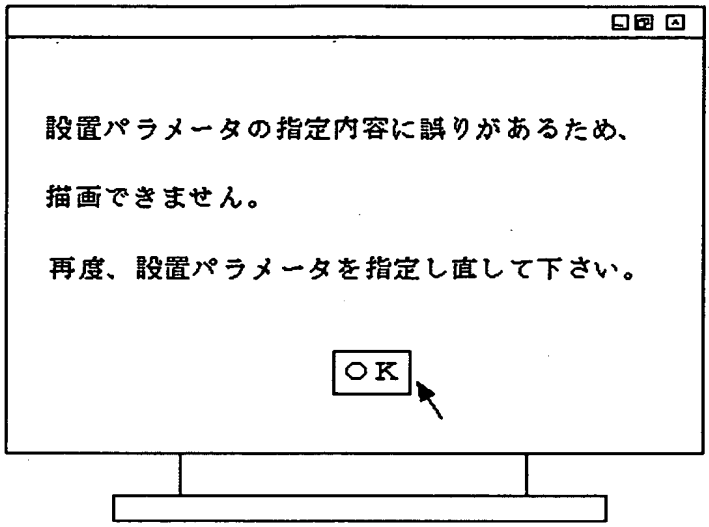

【図６】

（図６）
設置パラメータ表示領域
Ｒ１



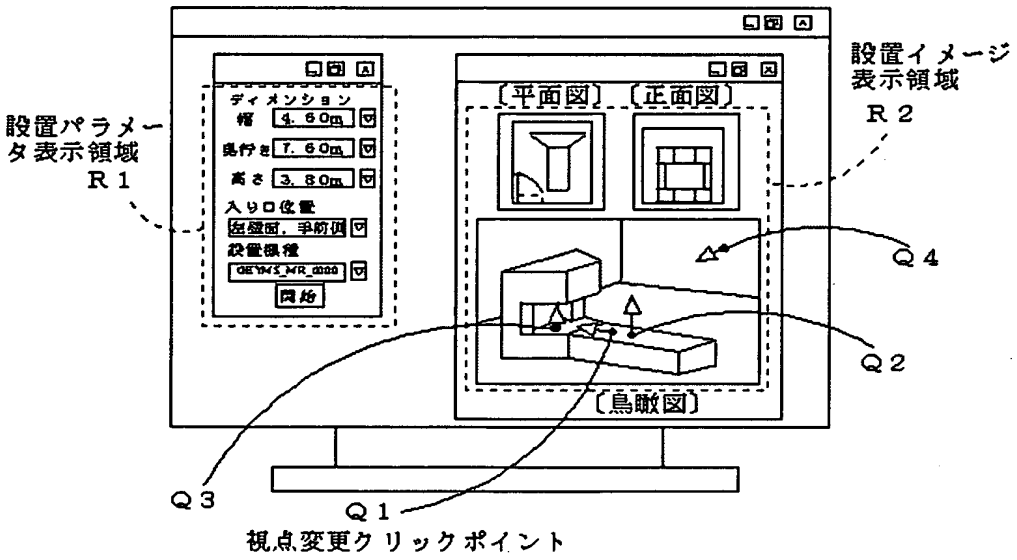
【図７】

（図７）　エラーメッセージ画面
Ｇ２

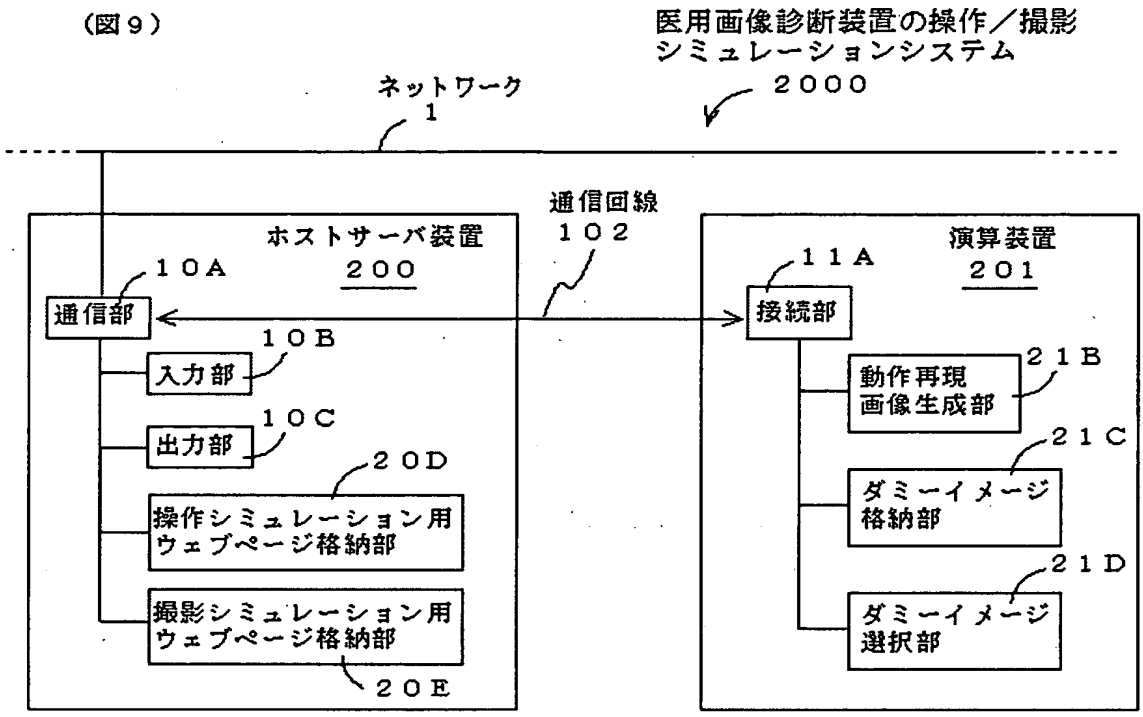



【図８】

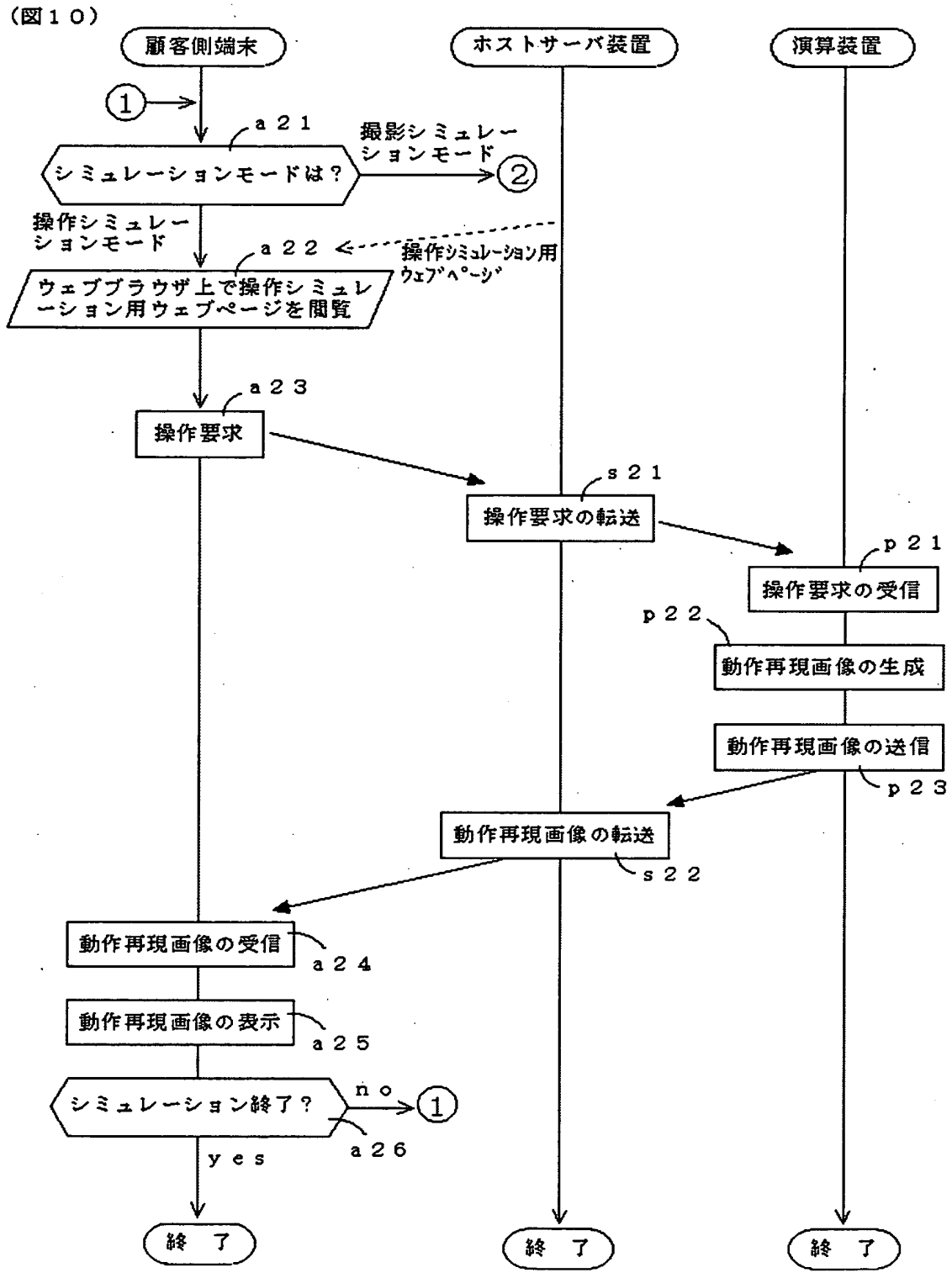
（図８）　設置シミュレーション用ウェブページ画面
Ｇ１'



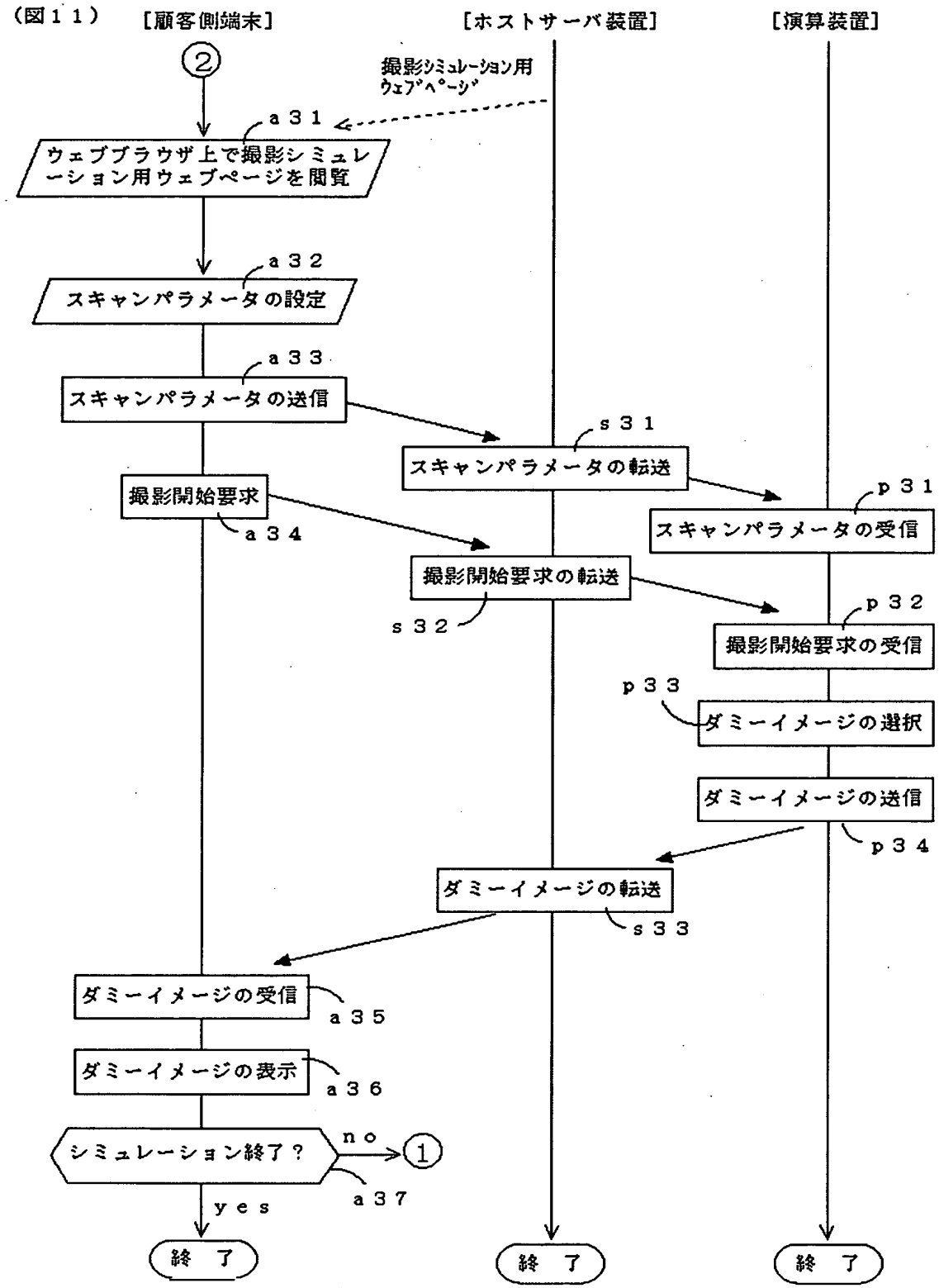
【図９】


（図９）
医用画像診断装置の操作／撮影
シミュレーションシステム
２０００
ネットワーク
１
通信回線
１０２
ホストサーバ装置
２００
１０Ａ
通信部
１０Ｂ
入力部
１０Ｃ
出力部
２０Ｄ
操作シミュレーション用
ウェブページ格納部
撮影シミュレーション用
ウェブページ格納部
２０Ｅ
演算装置
２０１
１１Ａ
接続部
２１Ｂ
動作再現
画像生成部
２１Ｃ
ダミーイメージ
格納部
２１Ｄ
ダミーイメージ
選択部

【図１０】


（図１０）
顧客側端末
ホストサーバ装置
演算装置
①
a２１
シミュレーションモードは？
撮影シミュレーションモード
②
操作シミュレーションモード
a２２
操作シミュレーション用ウェブページ
ウェブブラウザ上で操作シミュレーション用ウェブページを閲覧
a２３
操作要求
s２１
操作要求の転送
p２１
操作要求の受信
p２２
動作再現画像の生成
動作再現画像の送信
p２３
動作再現画像の転送
s２２
動作再現画像の受信
a２４
動作再現画像の表示
a２５
シミュレーション終了？
no
①
a２６
yes
終　了
終　了
終　了

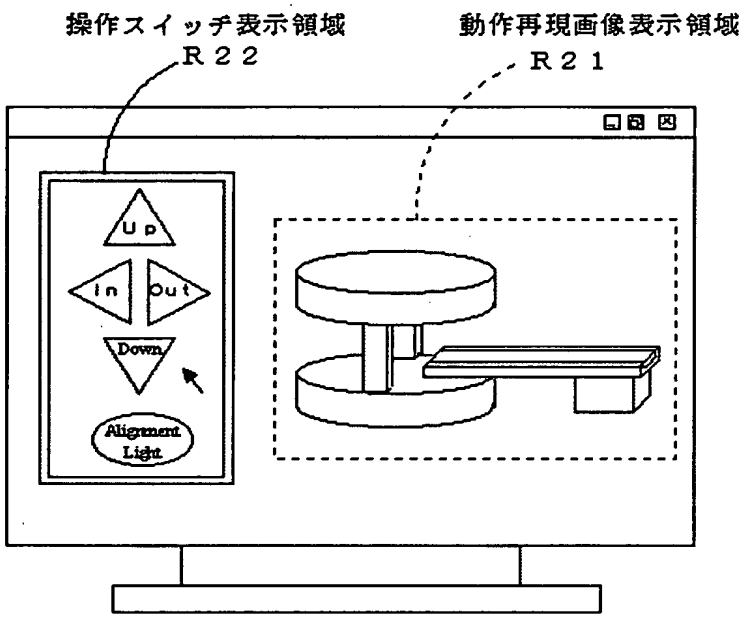
【図１１】


（図１１）
[顧客側端末]
[ホストサーバ装置]
[演算装置]
②
撮影ｼﾐｭﾚｰｼｮﾝ用
ｳｪﾌﾞﾍﾟｰｼﾞ
a３１
ウェブブラウザ上で撮影シミュレーション用ウェブページを閲覧
a３２
スキャンパラメータの設定
a３３
スキャンパラメータの送信
s３１
スキャンパラメータの転送
p３１
スキャンパラメータの受信
撮影開始要求
a３４
撮影開始要求の転送
s３２
p３２
撮影開始要求の受信
p３３
ダミーイメージの選択
ダミーイメージの送信
p３４
ダミーイメージの転送
s３３
ダミーイメージの受信
a３５
ダミーイメージの表示
a３６
シミュレーション終了？
no
①
a３７
yes
終 了
終 了
終 了

【図１２】

（図１２）

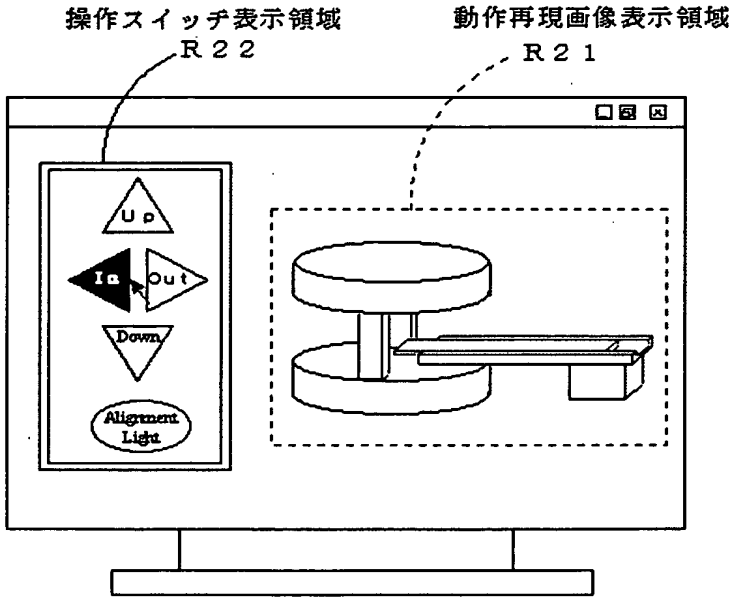
操作シミュレーション用ウェブページ画面
Ｇ２０




【図１３】

（図１３）

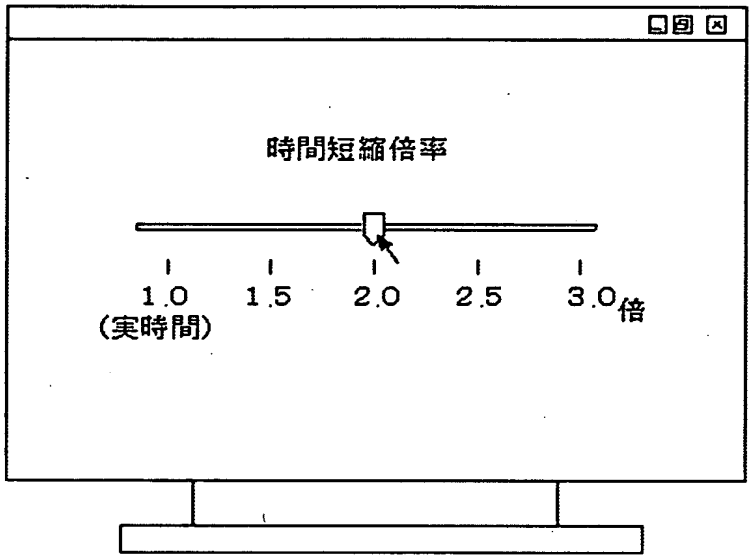
操作シミュレーション用ウェブページ画面
Ｇ２０'



【図１４】

（図１４）

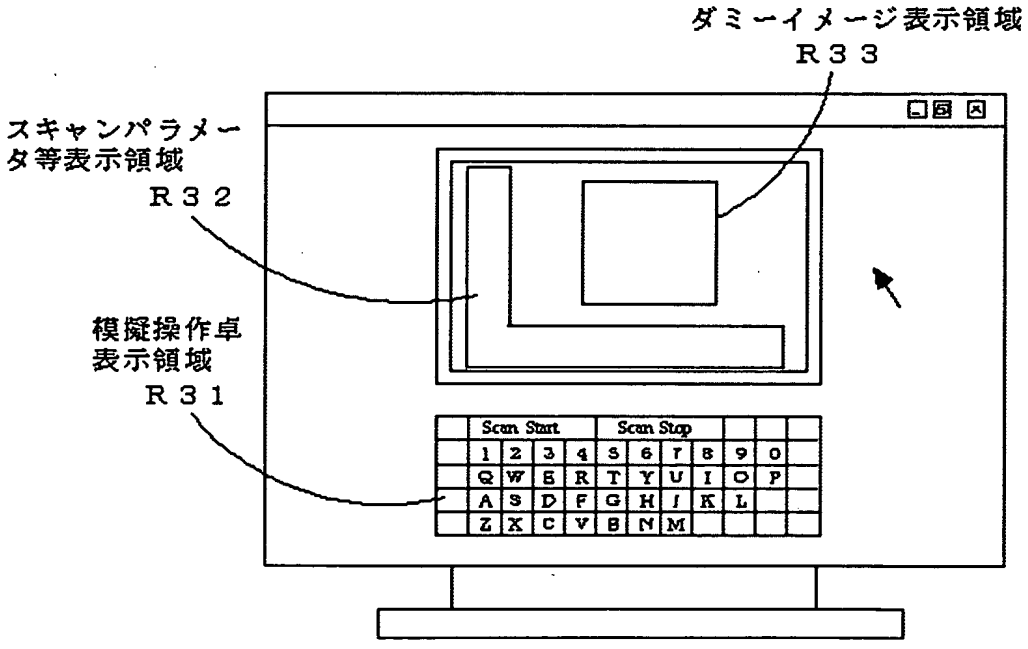
時間短縮倍率調整画面
Ｇ２１




【図１５】

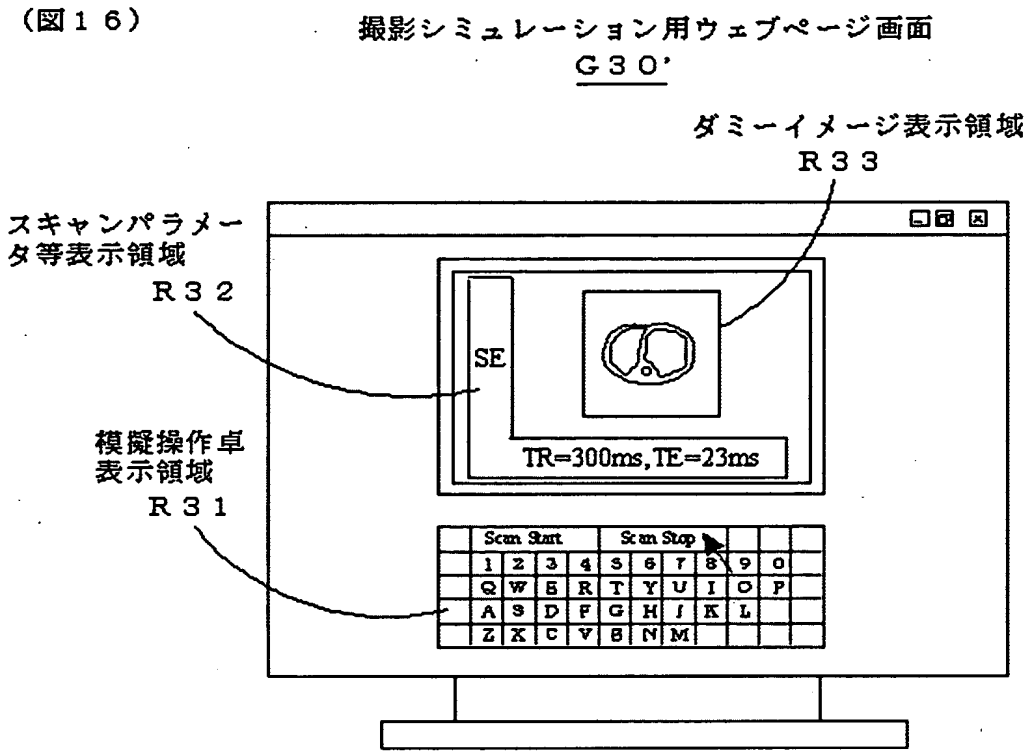
（図１５）

撮影シミュレーション用ウェブページ画面
Ｇ３０



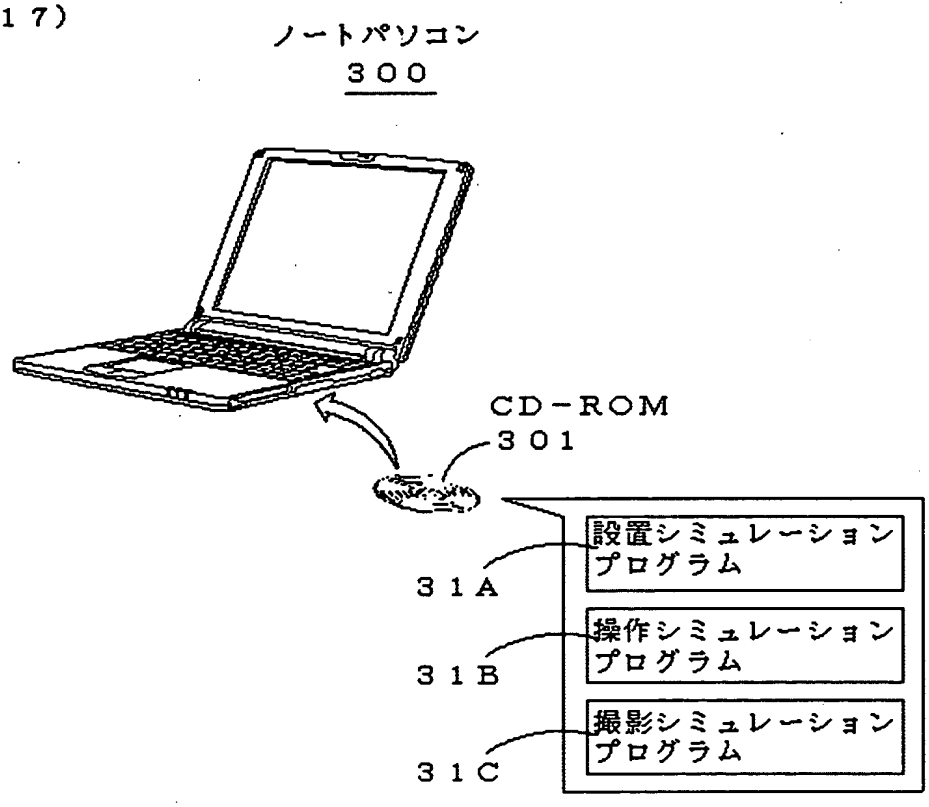
【図１６】

（図１６）



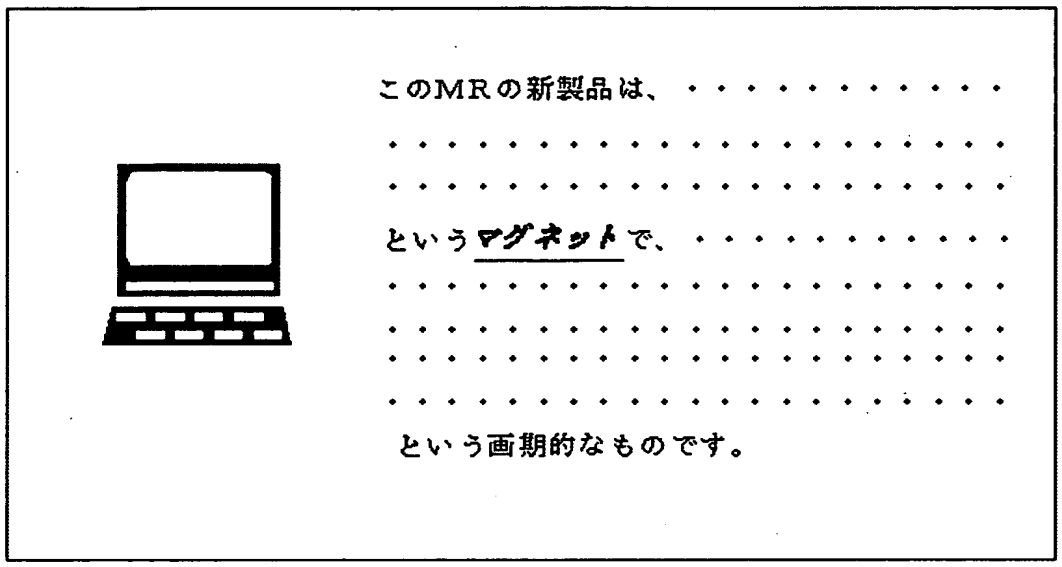

【図１７】

（図１７）



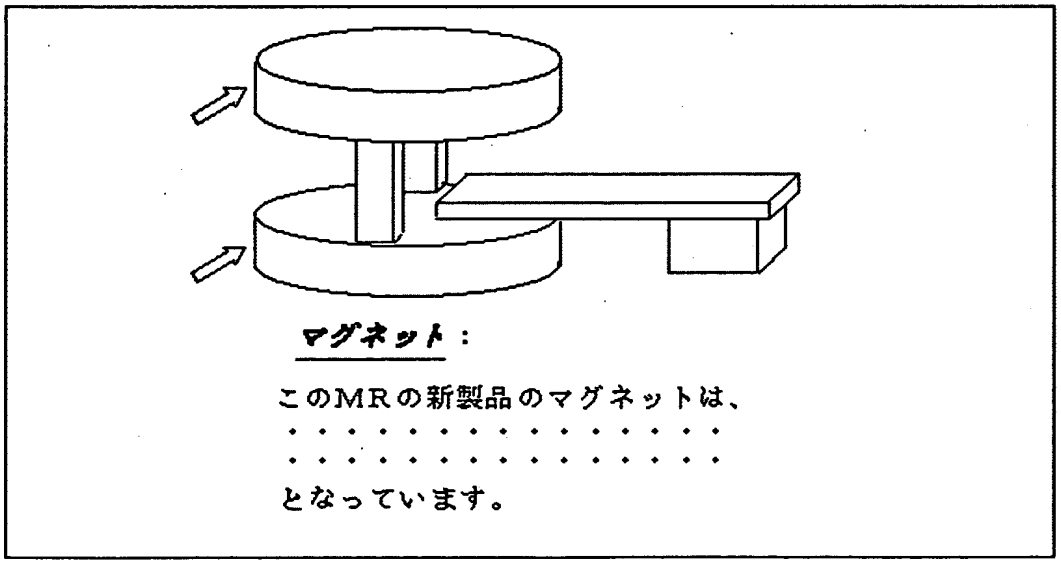
【図１８】

（図１８）




【図１９】

（図１９）



【書類名】　　要約書

【要約】

【課題】　医用画像診断装置の設置イメージを自動表示する。

【解決手段】　設置シミュレーション用ウェブページ画面Ｇ１’の設置パラメータ表示領域Ｒ１内で、撮影室の大きさ（幅，奥行き，高さ）と，医用画像診断装置の設置機種を指定すると、設置イメージ表示領域Ｒ２に、医用画像診断装置を撮影室内に仮想的に設置したときの設置イメージ（鳥瞰図，平面図，正面図）が自動表示される。視点変更クリックポイントＱ１～Ｑ４をクリックすると、視点を変更した設置イメージに更新される。

【効果】　指定した大きさの設置空間内に医用画像診断装置を設置した状況を表した設置イメージを自動表示できる。

【選択図】　　図８

# 出　願　人　履　歴　情　報

識別番号　　〔３００００１９２３８〕

１．変更年月日　　２０００年　３月１５日
［変更理由］　名称変更
住　所　　アメリカ合衆国・ウィスコンシン州・５３１８８・ワウケシャ・ノース・グランドヴュー・ブールバード・ダブリュー・７１０・３０００
氏　名　　ジーイー・メディカル・システムズ・グローバル・テクノロジー・カンパニー・エルエルシー

INHIBITING PRO-APOPTOTIC COUNTERPARTS OF Bcl-2

In general, BAX, BAK, Bcl-$X_S$ and others are pro-apoptotic proteins (Boise and Thompson, 1998). As set forth above, overexpression of BAX, BAK, Bcl-$X_S$ has been shown to override the incoming signals from cytokine-mediated signaling associated with cell viability and to induce apoptosis.

Accordingly, variant, non-functional human BAX, BAK, Bcl-$X_S$ genes may generated from the wild type BAX, BAK, Bcl-$X_S$ genes. Such mutant genes may be used to generate transformed cells deficient in BAX, BAK, or Bcl-$X_S$ activity, respectively, allowing for inhibition of apoptosis. The inhibitory effect of such the biologically inactive form of BAX, BAK, or Bcl-$X_S$ on apoptosis provides a means to circumvent the stimulatory effect of PKR overexpression on apoptotic cell death in cultured cell lines.

A mutated or variant human BAX, BAK, or Bcl-$X_S$ cDNA sequence may be inserted into a vector effective to express the inserted fragment under the control of a strong viral promoter, as described above.

Cells expressing a modified form of human BAX, BAK, or Bcl-$X_S$ are thereby generated, selected, further cultured in manner effective to result in production of a cytokine or other protein of interest, and then analyzed for the biosynthesis of the cytokine or other protein of interest, as described below.

V. CYTOKINES

Cytokines elicit their biological activities by binding to their cognate receptors followed by signal transduction leading to stimulation of various biochemical processes. In some cases, the expression of such receptors is regulated by specific signals, for example a cytokine may be involved in positive or negative feedback loops and thereby regulate the expression of the receptor for the same or a different cytokine. Such receptors may be the same type of cell that produces the cytokine or a different type of cell.

Cytokines serve to mediate and regulate immune and inflammatory responses. In general, cytokine production is transient and production takes place during a short period of transcription resulting in production of mRNA transcripts which are also short-lived and subject to post-transcriptional control mechanisms. Recent studies have indicated

that a common signal transduction pathway, the "Jak/STAT" pathway, is used by a variety of cytokines (Abbas, *et al.*, 1997).

It will be appreciated that the cellular source of cytokines is a distinguishing characteristic of each individual cytokine that may be produced by multiple diverse types of cells. In addition, a given cytokine (1) may act on more than one type of cells, (2) may have more than one effect on the same cell, (3) may have an activity shared with another cytokine, and (4) may influence the synthesis or effect of other cytokines, *for example*, by antagonizing, or synergizing the effects thereof.

The cytokine(s) produced may be one or more of the following: interferons, including IFN-gamma, IFN-alpha and IFN-beta; tumor necrosis factors (TNF), including TNF-alpha, TNF-beta and TNF soluble receptors (sTNF-R); interleukins (IL), including IL-2, IL-3, IL-4, IL-5, Il-6, Il-7, IL-8, IL-11 and IL-12; colony stimulating factors, including granulocyte colony stimulating factors (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF); angiogenic factors, including fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF); platelet-derived growth factors 1 and 2 (PDGF 1 and 2); chemokines, including Regulated Upon Activation Normally T-Expressed Secreted (RANTES); macrophage inflammatory proteins (MIP), such as MIP-1alpha and MIP-2alpha; monocyte chemoatactic proetin-1(MCP); anti-angiogenic factors, including angiostation; endostatin leukemia inhibitory factor (LIF); ciliary neurotrophic factor; cardiotrophin and oncostatins, including oncostatin M.

The methods of the invention may also be used to increase the expression of any of a number of proteins which are capable of production in cell culture. Exemplary proteins include, but are not limited to, insulin, erythropoietin (EPO), tissue plasminogen activator (TPA), growth hormone and Factor VIII.

Once increased expression of a given cytokine or other protein is achieved, the cytokine or other protein thereby produced is purified from the cell culture. Exemplary procedures suitable for such purification include the following: antibody-affinity column chromatography, ion exchange chromatography; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; and gel filtration using, for example, Sephadex G-75. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, 1990;

Scopes, 1982. The purification step(s) selected will depend, for example, on the nature of the production process used and the particular cytokine or protein produced.

A "higher than normal level" of cytokine or other protein production means at least 200 or 300%, preferably 500% or more, of the cytokine or other protein production level for a given cell line in the absence of either transforming the cell line in a manner effective to result in over-expression of PKR or modifying the cell line in a manner effective to inhibit apoptotic cell death.

In the methods of the invention, it is preferred that a human cell line is modified by the combination of PKR overexpression and inhibition of apoptosis or inhibition of apoptosis alone, and cultured in a manner effective to enhance cytokine or other protein production respectively, by 10-1000 fold.

## VI. PKR OVEREXPRESSION, INHIBITION OF APOPTOSIS AND CYTOKINE PRODUCTION

A number of factors are known to be involved in the induction and/or enhanced expression of cytokines in cells, e.g., human cells. These factors include cytokine- and other protein-specific transcriptional regulators, for example interferon regulatory factors (IRF-1, IRF-3 and IRF-7), cytokine receptors, nuclear factor κB (NF-κB), activator protein-1 (AP-1), nuclear factor IL-6 (NF-IL6), and in particular PKR.

Enhancing the expression or activity of any of these factors will result in a higher than normal level of expression of the genes which encode one or more cytokines. Such enhanced expression of cytokine genes will result in more efficient and lower cost production of cytokines.

PKR, is used as herein as an example of a protein capable of regulating cytokine and other protein expression; however, it will be understood that other cytokine and other protein enhancing factors may be used in place of PKR, *for example*, 1) protein kinase C (PKC) inducers, TNF-α, GM-CSF, EGF and PDGF, G-CSF, TGF, TNF-alpha or TNF-beta, IL-1, IFNs (IFN-alpha, IFN-beta, IFN-gamma) or chemokines (IL-8, Macrophage inflammatory proteins [MIP-1a & -1b] and monocyte chemotactice proteins [MCPs]); 2) other cellular signaling factors such as PMA, calcium ionophores, sodium butyrate or endotoxin ; 3) polyI: C, double-stranded RNA or viral analog; 4) cellular stress signals that can activate PKR including heat shock or pathogen infections including virus), overproduces activated PKR and various cytokines.

By increasing the expression of PKR in a human cell, cytokine production can be increased. Animal cell cultures which express a higher than normal constitutive level of PKR or in which PKR expression can be induced to higher than normal levels are therefore useful for the production of cytokines.

The cells used to produce a given cytokine can overexpress PKR from any mammalian source, such as the PKR normally found in rabbit reticulocytes, various mouse tissues, or human peripheral blood mononuclear cells. Preferably murine p65 kinase and most preferably human p68 kinase is overexpressed, in a corresponding murine or human cell culture, respectively.

In some cases, the PKR which is overexpressed is an analog of PKR, *for example*, a non-natural protein kinase that can mediate dsRNA activation of cytokine and other protein transcription (usually obtained by modification of the gene encoding a native PKR protein).

Human cells capable of overexpressing PKR may be obtained by any number of methods, that are well known in the art or may be obtained from commercial sources.

Exemplary methods for obtaining PKR-overexpressing cells include selection for cells expressing higher than normal PKR levels, transfection with an expression vector encoding PKR under control of a promoter, or other methods which result in an increase in PKR expression over normal levels.

Appropriate promoters for use in such expression vectors include both constitutive promoters and inducible promoters, examples of which include a CMV promoter, and the metallothienein promoter.

Transfection is carried out as previously described and transfectants are selected for over-expression of PKR.

By over-expression of PKR is meant higher than normal levels of PKR activity. Such "normal" PKR activity or expression is reported as a range of PKR activity or expression, which is generally observed for a given type of cells which have not been transfected with a vector encoding PKR, are unstimulated (not induced or primed) and uninfected. It will be understood that the range of normal PKR activity for a given type of cell may vary somewhat dependent upon culture conditions.

Higher than normal PKR expression means at least 150%, preferably at least 200 or 300%, and more preferably 500% or more, of the normal PKR level. The PKR-

overexpressing cell culture may be constitutive for PKR over-expression or inducible for PKR over-expression, depending on the particular method used to isolate or prepare the culture.

Preferably the PKR-overexpressing cell line will be inducible for PKR over-expression in order to regulate the level of PKR available for cytokine induction.

Similarly, preferably the cell culture will be inducible for overexpression of a protein which interferes with the apoptotic process or for the expression of a modified form of a protein which facilitates the apoptotic process, in order to regulate the apoptotic process in conjunction with PKR expression for optimal cytokine induction.

The activity of PKR and apoptosis-associated proteins may be determined by any of the methods known in the art. Exemplary assays for PKR expression include autophosphorylation assays, assay for eIF2$\alpha$, Western blot, and (reverse transcriptase polymerase chain reaction) RT-PCR for PKR mRNA. Similarly, the expression of apoptosis-associated proteins may be determined by Western blot, and RT-PCR.

Any of a number of known cell types, modified in a manner to inhibit apoptosis, are useful for making a PKR-overexpressing cell line.

Any of a number of known cell cultures are useful as a parental strain for making a PKR-overproducing cell culture. Any cells normally capable of producing cytokines are suitable as the parental strain, as noted above. However, any cell line capable of producing a given cytokine or protein of interest may be employed in the methods of the invention. Human cell lines capable of cytokine or other protein production may be obtained by any number of methods that are well known in the art, including isolation of primary cell lines, or such cell lines may be obtained from commercial sources. In most cases, cells capable of producing a given cytokine or other protein are cultured in any suitable medium.

In some cases, additional steps are taken to enhance PKR expression by human cells, particularly, priming the PKR-expressing cells. Such priming may include treating with a priming agent, such as 1) G-CSF, EGF, TNF-alpha or TNF-beta, IL-1, interferons including IFN-alpha, IFN-beta, IFN-gamma or chemokines including IL-8, macrophage inflammatory proteins including MIP-1a & -1b and monocyte chemotactice proteins (MCP); 2) other cellular signaling factors such as phorbol myristate acetate (PMA), calcium ionophores, sodium butyrate or endotoxin; 3) poly IC, double-stranded RNA or viral

analog; 4) cellular stress signals that can activate PKR including heat shock or pathogen infections including virus.

Such treating may include adding a microbial or non-microbial inducer to the cell culture. Preferably, the inducer will be a non-microbial inducer, *for example*, poly IC or poly rIC.

## VII. EVALUATION OF CYTOKINE OR OTHER PROTEIN EXPRESSION

In order to evaluate the expression of a cytokine or other protein of interest by a PKR-overexpressing cell line, which has been treated in manner effective to inhibit apoptosis, assays can be carried out at the protein level, the RNA level or by use of functional bioassays particular to the individual cytokine or other protein being expressed.

To demonstrate the invention, cells lines transfected with a PKR gene, and with both PKR and $BclX_L$ genes were tested for cell viability under conditions of cytokine induction, with both polyIC and Sendai virus dsRNA, as detailed in Example 3. In these studies, "6A" cells were transfected with both PKR and Bcl-$X_L$ genes; "A9" cells, with the PKR gene only; and "WT", non-transformed. The cells were tested under conditions of PKR overproduction (which would occur in the 6A and A9 cells), following cytokine induction with either polyIC or Sendai virus RNA. As seen from the data in Figs. 2A and 2B, inhibiting apoptosis in PKR overproducing cells significantly increased cell viability under conditions of cytokine induction, and even enhanced viability over WT cells (no PKR overproduction).

In a related experiment, also detailed in Example 3, expression levels of IFN-alpha were measured in the same three cells lines, again under conditions of PKR overproduction and cytokine induction with either Sendai virus or polyIC. From the data in Fig. 3A, it is seen that PKR overproduction (6A and A9 vs WT) significantly enhances cytokine production, and that a several fold further enhancement in cytokine production was observed by inhibiting apoptosis during cytokine-induction conditions (6A vs. A9). The analogous results in Fig. 3B also illustrate the significant enhancement in cytokine induction achieved with PKR overproduction (6A and A9 vs. WT). The higher levels of cytokine production observed in A9 vs. 6A cells may reflect a temporal effect and does not consider the overall amount of cytokine production during the period of cell viability.

Immunoassays for a particular cytokine or other proteins may be carried out using procedures routinely employed by those of skill in the art. Such immunoassays can be used to qualitatively and quantitatively analyze expression of a cytokine or other protein of interest.

In general, a purified form of the cytokine or other protein of interest, is either obtained from a natural source or produced recombinantly in transfected cells, and purified using standard techniques for protein purification. The purified protein is then used to produce either monoclonal or polyclonal antibodies specific to the expressed protein, and which can be used in various immunoassays. (See, *for example*, Harlow and Lane, 1988). Exemplary assays include ELISA, competitive immunoassays, radioimmunoassays, Western blots, indirect immunofluorescent assays and the like.

In general, kits which are commercially available may be used for the quantitative immunoassay of the expression level of known cytokines or other proteins.

In addition, the functional expression of eukaryotic proteins is well known. Exemplary methods are described in Sambrook, *et al.*, 1989, expressly incorporated by reference herein. Briefly, cells are transfected with a suitable expression vector and cultured under conditions effective to result in expression of the cytokine or other protein of interest into the culture medium or on the surface of the transfected cell.

Specific examples are described above, however, it will be apparent to one of ordinary skill in the art that many modifications are possible and that the examples are provided for purposes of illustration only and do not limit the invention, unless so specified.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

## Example 1

### Preparation of Plasmids pEF-FLAG-Bcl-$X_L$ and pcDNA-FLAG-PKR

1. Preparation of pEF-FLAG-Bcl-$X_L$

The pEF-FLAG-Bcl-$X_L$ vector (Huang, et al., 1997) in Figure 1A contains a full length cDNA encoding the anti-apoptotic Bcl- $X_L$ protein operably linked to the strong elongation factor 1alpha (EF-1alpha) promoter. An additional salient

feature of the vector is the N-terminal FLAG epitope (Hopp et al., 1988) that was added to the Bcl-$X_L$ protein to facilitate selection of cell lines that express high levels of Bcl-$X_L$.

The vector also includes i) a polyadenylation signal and transcription termination sequence to enhance mRNA stability; ii) a SV40 origin for episomal replication and simple vector rescue; iii) an ampicillin resistance gene and a ColE1 origin for selection and maintenance in E. coli; and iv) a puromycin resistance marker (Puro) to allow for selection and identification of the plasmid-containing eukaryotic cells after transfection of a Bcl-$X_L$ and PKR.

2. Preparation of pcDNA-FLAG-PKR

The pcDNA-FLAG-PKR vector in Figure 1B contains cDNA encoding the full-length human PKR molecule (551 amino acids; Meurs, et al., 1990; GenBank Accession No. NM002759) modified by the polymerase chain reaction to include the N terminal FLAG tag (Hopp et al.,1988) encoding the sequence MDYKDDDDK, and inserted into the eukaryotic expression vector pcDNA3 (Invitrogen), such that the FLAG-PKR coding sequence was expressed under the control of the CMV promoter.

The vector, termed pcDNA-FLAG-PKR, contains various features suitable for PKR transcription, including: i) a promoter sequence from the immediate early gene of the human CMV for high level mRNA expression; ii) a polyadenylation signal and transcription termination sequence from the bovine growth hormone (BGH) gene to enhance mRNA stability; iii) a SV40 origin for episomal replication and simple vector rescue; iv) an ampicillin resistance gene and a ColE1 origin for selection and maintenance in E. coli; and v) a G418 resistance marker (Neo) to allow for selection and identification of the plasmid-containing eukaryotic cells after transfection.

A second PKR vector, designated pTRE-PKR, was prepared by inserting the same PKR cDNA into the gene-insertion site of a pTRE plasmid obtained from Clonetech. The pTRE plasmid is similar to the pFLAG used in making the first-described PKR vector, but contains a tetracycline-responsive element upstream of the CMV promoter used to control the inserted gene. In the studies

reported in Example 3, the TRE function was not exploited, and so the operation of the two PKR vectors in transformed cells is expected to be essentially identical.

Example 2

Preparation of PKR Over-producing Namalwa Cell Lines 6A and A9

1. Preparation of Cell Line 6A

The human B lymphoblastoid cell line Namalwa (WT) was transfected sequentially with the plasmids, pEF-FLAG-Bcl-$X_L$ and pcDNA-FLAG-PKR. The transfected cell line is termed 6A.

Stable transfectants were obtained by electroporation of $4x10^6$ exponentially growing Namalwa cells with 15ug of the pEF-FLAG-Bcl-$X_L$ plasmid in DMEM/F12 (+10% FBS) using a Gene Pulser apparatus (BioRad) set at 800 uF, 300V. Bulk populations of stable transformants were obtained by selection with 2 ug/ml puromycin (Gibco-BRL) for 3-4 weeks and screened for Bcl-$X_L$ expression by flow cytometry as follows. The bulk transfectants were washed, permeabilized with acetone and subsequently stained with 2 ug/ml mouse anti-FLAG M2 monoclonal antibody (IBI) and then with phycoerythrin conjugated goat anti-mouse IgG (1ug/ml; Becton-Dickinson). Cells were analyzed in the FACScan, live and dead cells being discriminated on the basis of their forward and side light-scattering properties and Bcl-$X_L$ expressing cells by their level of fluorescence intensity. High level Bcl-$X_L$ expressing transformants (Namalwa-Bcl-$X_L$) were then transfected with pcDNA-FLAG-PKR.

Stable high level Bcl-$X_L$ expressing transfectants were obtained by electroporation of $4x10^6$ exponentially growing Namalwa- Bcl-$X_L$ cells with 15 ug of the pcDNA-FLAG-PKR plasmid in DMEM/F12 (+10% FBS) using a Gene Pulser apparatus (BioRad) set at 800uF, 300V. Bulk populations of stable transformants were obtained by selection with 2 mg/ml geneticin (G418, Gibco-BRL) for 3-4 weeks. Clonal lines were subsequently obtained by limiting dilution cloning and analyzed for Bcl-$X_L$ and PKR expression by Western blot analysis (Huang et al.,1997). The proteins were identified using 2 ug/ml anti-

FLAG M2 antibody followed by goat anti-mouse IgG-peroxidase conjugate and ECL detection (Amersham).

2. Preparation of Cell Line A9

Stable high level PKR expressing transfectants were obtained by electroporation of $4x10^6$ exponentially growing Namalwa cells with 15 ug of the pTRE-PKR plasmid in DMEM/F12 (+10% FBS) using a Gene Pulser apparatus (BioRad) set at 800uF, 300V. Bulk populations of stable transformants were obtained by selection with 2 mg/ml geneticin (G418, Gibco-BRL) for 3-4 weeks. Clonal lines were subsequently obtained by limiting dilution cloning and analyzed for PKR expression by Western blot analysis (Huang et al.,1997).

Example 3

Characterization of a Bcl-$X_L$ and PKR Over-producing Namalwa Cell Line

1. Increased Cell Viability

Wildtype Nowalwa cells (WT) and the A9 and 6A cells from Example 2 were examined for cell viability in culture under conditions of PKR overproduction and cytokine induction. Specifically, PKR and Bcl-$X_L$ double-transfected Namalwa cells (the 6A cell line), PKR-transfected Namalwa cells (the A9 cell line) and parental Namalwa cells (WT) were cultured at $2.5x10^5$ cells/ml in DMEM/F12 medium supplemented with 10% FBS. The cells were treated with 20 mM PMA (primer) for 20 hr followed by treatment with either 200 ug/ml poly r(I):poly r(C) and 10 ug/ml DEAE Dextran (poly IC induction) for 72 hr. or 200 HAU/$1x10^6$ cells of Sendai virus for 48 hr. Following treatment, cell viability was assessed by flow cytometry on a FACScan.

Figures 2A and 2B show that poly IC induction of all three cell lines resulted in significantly less cell viability than Sendai virus induction of the cells at the respective, indicated time periods. With poly IC induction, 54% of 6A, 40% of A9 and 51% of WT cells remained viable, whereas with Sendai virus induction, 87% of 6A, 66% of A9 and 63% of WT cells remained viable.

With both induction protocols, the 6A cell line, which overexpresses both the anti-apoptotic protein Bcl-$X_L$ and PKR, showed greater viability than the A9 cell line which overexpresses PKR, but is not inhibited for apoptosis.

2. Increased Expression of Interferon-alpha

The level of IFN-alpha production was also analyzed in the three cell lines following cytokine induction by poly IC and Sendai virus, both under conditions of PKR overproduction. The culture supernatants were collected and analyzed for IFN-alpha levels by ELISA according to the procedure provided by the supplier of the ELISA kits (R&D Systems). The results are shown in Fig. 3A and 3B, and discussed above.

From the foregoing, it can be seen how various objects and features of the invention are met. Those skilled in the art can now appreciate from the foregoing description that the broad teachings of the present invention can be implemented in a variety of forms. Therefore, while this invention has been described in connection with particular embodiments and examples thereof, the true scope of the invention should not be so limited. Various changes and modification may be made without departing from the scope of the invention, as defined by the appended claims.

IT IS CLAIMED

1. A method for producing cytokines in a human cell culture, comprising:

(a) culturing a human cell line capable of producing cytokines and transfected with (i) a first vector containing DNA encoding a protein effective to inhibit cell apoptosis under the control of a first promoter; and (ii) a second vector containing DNA encoding double-stranded-RNA-dependent-kinase (PKR) under the control of a second promoter, under culture conditions in which PKR is overproduced in the transfected cells, as evidenced by levels of PKR in the transfected cell line which are higher than those obtained in the human cell line which is not transfected with the first and second vectors, when grown under the same culture conditions,

(b) treating the cultured, PKR overproducing human cell line with double-stranded RNA (dsRNA), and

(c) collecting one or more cytokines produced by the cultured, treated cell line.

2. The method of claim 1, wherein the cultured cell line is prepared by transfecting a human cell capable of producing cytokines successively with the first vector and the second vector.

3. The method of claim 1 or 2, wherein the protein effective to inhibit apoptosis is selected from the group consisting of B-cell Lymphoma/Leukemia-2 gene (Bcl-2a), B-cell Lymphoma/Leukemia- $X_L$ (Bcl-$X_L$), a modified form of eukaryotic translation initiation factor 2 alpha (eIF-2 alpha), eukaryotic translation initiation factor (eIF-3), a modified form of Fas-associated death domain (FADD), a modified form of Bcl-$X_S$, a modified form of Bcl-2-homologous anagonist/killer (BAK) and a modified from of BAX.

4. The method of claim 3, wherein the protein effective to inhibit apoptosis is Bcl-2a or Bcl-$X_L$.

5. The method of claim 1, wherein the first or second promoter is an inducible promoter.

6. The method of claim 5, wherein the inducible promoter is a metallothionein promoter.

7. The method of claim 1, wherein the cytokine(s) produced are selected from the group consisting of:

i) interferons selected from the group consisting of IFN-alpha and IFN-beta, IFN-gamma;

ii) tumor necrosis factors (TNF) selected from the group consisting of TNF-alpha, TNF-beta and TNF soluble receptors (sTNF-R);

iii) interleukins (IL) selected from the group consisting of IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-11 and IL-12;

iv) colony stimulating factors selected from the group consisting of granulocyte colony stimulating factors (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF);

v) angiogenic factors selected from the group consisting of fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet-derived growth factors 1 and 2 (PDGF 1 and 2);

vi) chemokines selected from the group consisting of Regulated Upon Activation Normally T-Expressed Secreted (RANTES), macrophage inflammatory proteins (MIP) including MIP-1alpha and MIP-2alpha and monocyte chemoatactice proetin-1 (MCP);

vii) anti-angiogenic factors selected from the group consisting of angiostation and endostatin;

viii) leukemia inhibitory factor (LIF);

ix) ciliary neorotrophic factor and cardiotrophin; and

x) oncostatins, including oncostatin M.

8. The method of claim 1, wherein the cultured human cell line is derived from a parental strain cell line selected from the group consisting of fibroblasts or immune cells, B cells, T cells, monocytes, neutrophils, natural killer cells, pro-monocytic U937 cells, Namalwa cells, MRC-5 cells, WI-38 cells, Flow 1000 cells, Flow 4000 cells, FS-4, FS-7

cells, MG-63 cells, CCRF-SB cells, CCRF-CEM, Jurkat cells, WIL2 cells and THP-1 cells.

9. In a method for producing cytokines in a human cell culture by culturing a human cytokine-producing cell under conditions of PKR overproduction and cytokine induction, an improvement for increasing the viability of the cells comprising employing as the cell line, cells which have been transfected with a vector containing DNA encoding a protein effective to inhibit apoptosis in the cells.

10. The improvement of claim 9, wherein the cell line is also transfected with a vector containing DNA expressing PKR.

11. The improvement of claim 9, wherein the DNA encoding a protein effective to inhibit apoptosis in the cells encodes a protein selected from the group consisting of Bcl-2, Bcl-$X_L$, a modified form of eukaryotic translation initiation factor 2 alpha (eIF-2 alpha), eukaryotic translation initiation factor (eIF-3), a modified form of Fas-associated death domain (FADD), a modified form of Bcl-$X_S$, a modified form of BAK and a modified from of BAX, operably linked to a second promoter, under conditions effective to result in expression of the protein by the cells of the transfected cell line.

12. The method of claim 11, wherein the protein effective to inhibit apoptosis is Bcl-2a or Bcl-$X_L$.

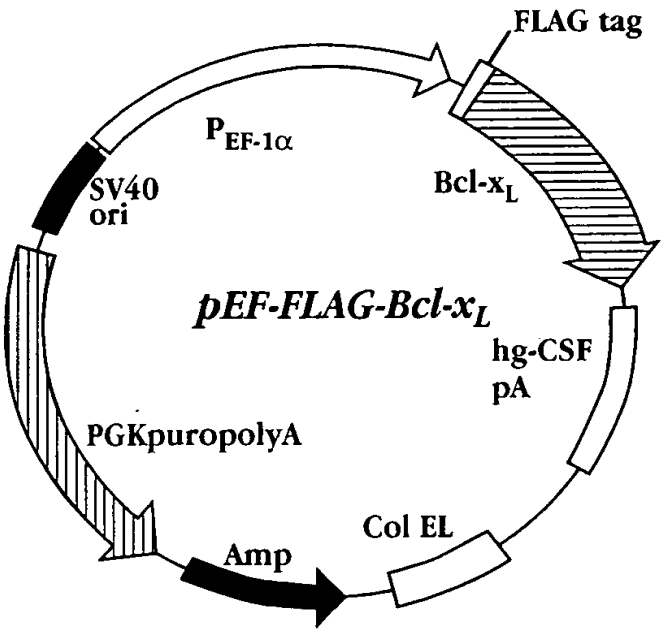



Fig. 1A

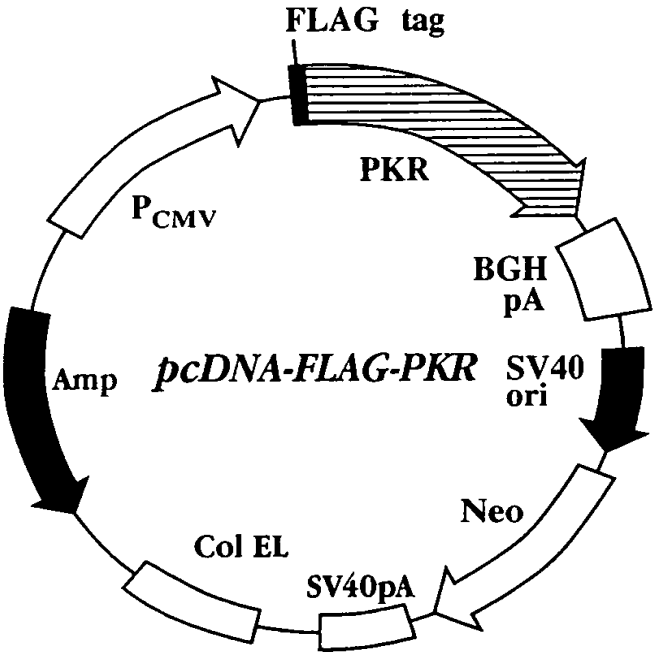



Fig. 1B

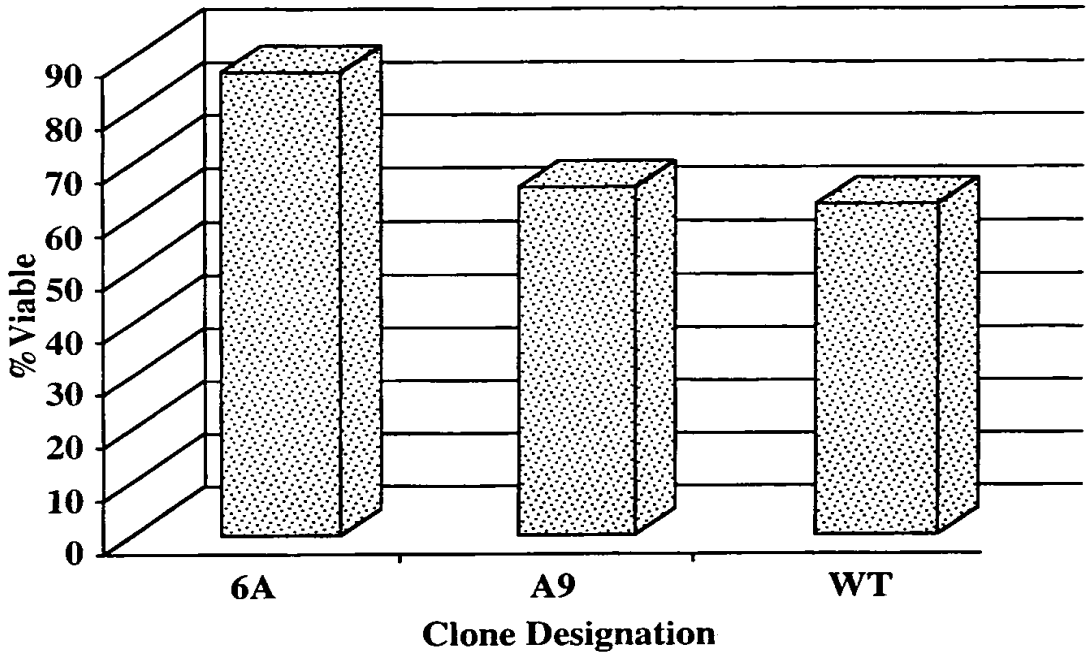



Fig. 2A

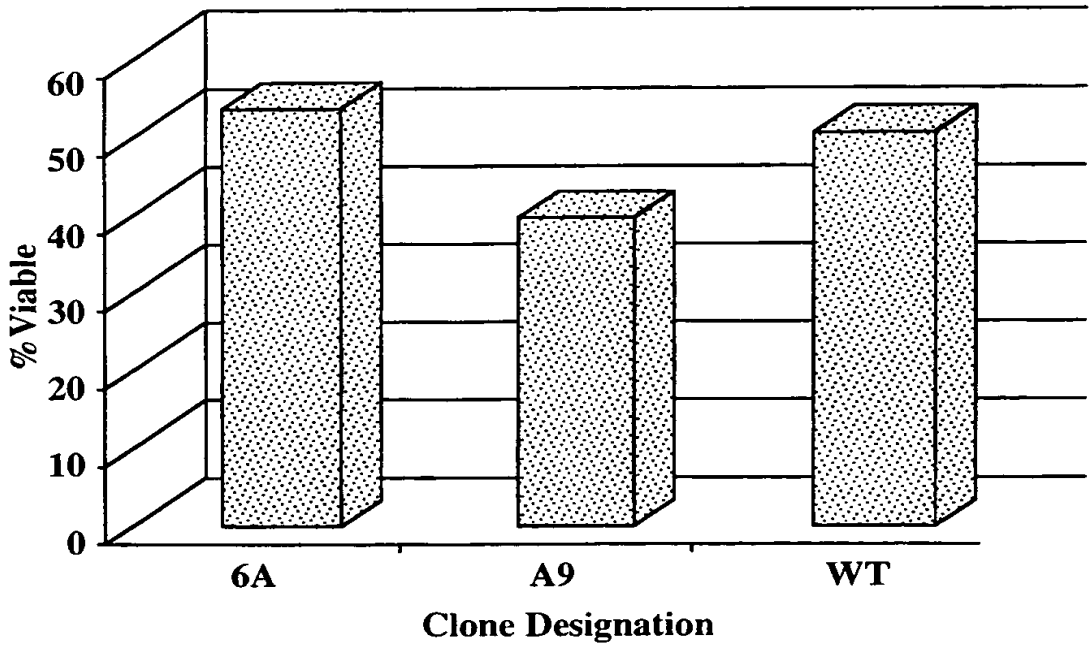



Fig. 2B

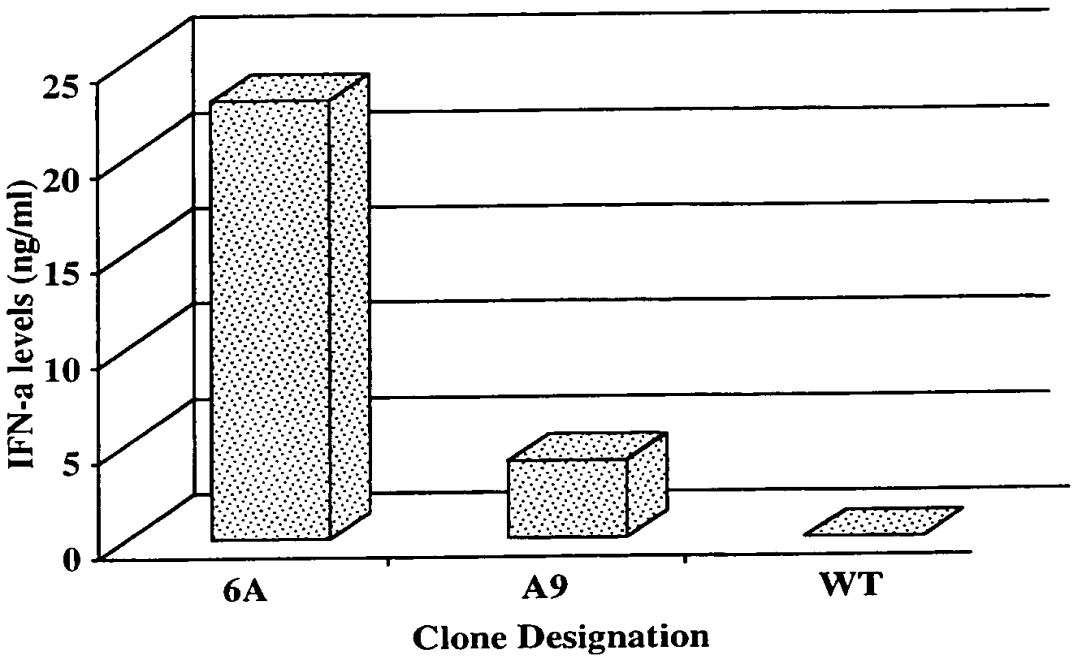



Fig. 3A

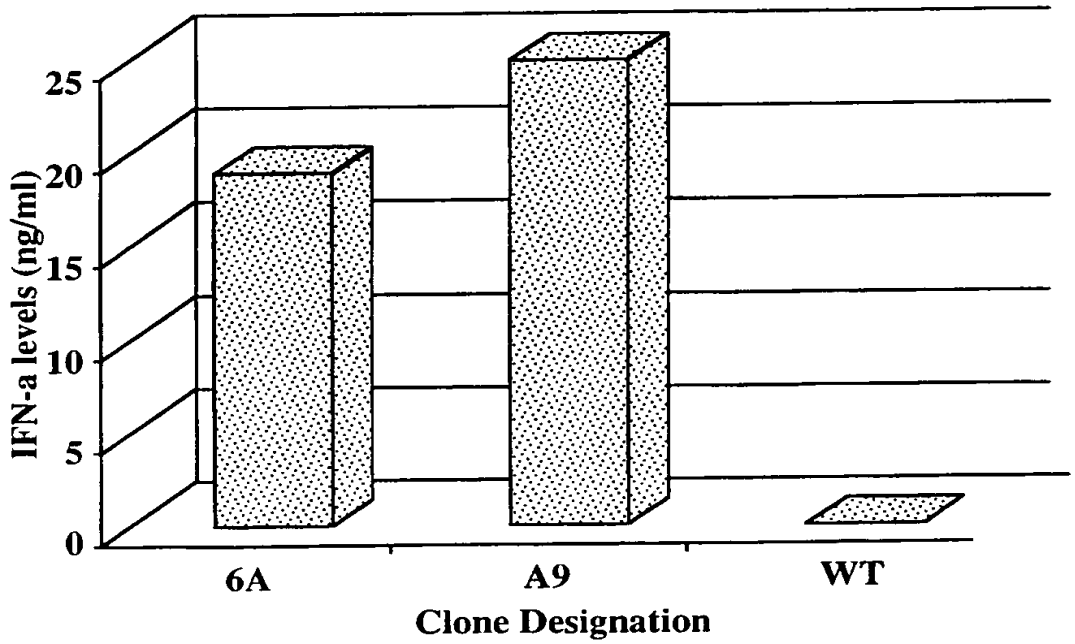



Fig. 3B

# INTERNATIONAL SEARCH REPORT

Int. :tional Application No
PCT/US 00/24657

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/12 C07K14/555

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 5 February 2001 | 19/02/2001 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31–70) 340–2040, Tx. 31 651 epo nl,<br>Fax: (+31–70) 340–3016 | Sprinks, M |

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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